(17) Uveitis

Original Paper

Ophthalmic Research

Ophthalmic Res 2001;33:251–255

Received: June 7, 2000
Accepted after revision: March 7, 2001

Aqueous Humor and Serum Tumor Necrosis Factor-α in Clinical Uveitis

Manuel Santos Lacomba[a] Carmen Marcos Martín[a]
José María Gallardo Galera[a] Maria Amparo Gómez Vidal[d]
Eduardo Collantes Estévez[b] Rafael Ramírez Chamond[c] M. Omar[d]

[a]Department of Ophthalmology, [b]Department of Rheumatology, and [c]Department of Experimental Medicine, Reina Sofia University Clinic, Cordoba, and [d]Department of Internal Medicine, Ciudad de Jaen University Clinic, Jaen, Spain

Key Words
Aqueous humor · Cytokines · Tumor necrosis factor-α · Uveitis, clinical

Abstract
Objective: To study the local and systemic behavior of the tumor necrosis factor-α (TNF-α) in patients with active uveitis. ***Methods:*** TNF-α levels were measured in aqueous humor and peripheral blood samples using an enzyme-linked immunosorbent assay from 23 patients with uveitis and 16 control patients who had been operated on for uncomplicated cataracts. ***Results:*** Aqueous humor and sera of patients with uveitis showed higher levels of TNF-α than those of controls ($p < 0.001$). A comparison of cytokine levels between aqueous humor and sera showed significantly higher levels of TNF-α in serum than aqueous humor ($p < 0.001$). Correlation studies using the regression test for successive steps showed that serum TNF-α levels correlated with recurrent uveitis ($r = 0.4150$; $p = 0.0489$). ***Conclusions:*** TNF-α is a cytokine that participates actively in the pathogenesis of clinical uveitis. Our data emphasize the greater systemic than local participation of TNF-α. Finally, an elevated serum TNF-α seems to be associated with a recurrent pattern of uveitis.

Introduction

Although the precise pathogenic mechanisms underlying uveitis have not been fully identified, cytokines appear to be involved in intraocular inflammation. Raised levels of several cytokines have been reported in serum and aqueous humor from patients with uveitis [1–3]. While some cytokines such as IL-6 have been extensively studied in human uveitis [4, 5], others, such as tumor necrosis factor-α (TNF-α), have been the subject of fewer studies [6–8].

TNF-α is an important mediator in metabolic and immunological responses and may be regarded as one of the earliest and most critical mediators in inflammation. TNF-α is synthesized by monocytes, macrophages, neutrophils, mast cells, natural killer and T lymphocytes [9]. Several studies have indicated that TNF is involved in the development of various experimental models of uveitis. Thus, analysis of endogenous TNF-α during endotoxin-induced uveitis (EIU) in rats shows an early rise in levels in the aqueous humor and serum [10, 11]. Moreover, intravitreal injection of TNF-α in rabbits [12] and in rats [13] induces acute uveitis characterized by an increase in aqueous humor protein and an infiltrate of polymorphonuclear granulocytes in the anterior chamber; these findings suggest that TNF-α may be an initiating factor in the

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–3747/01/0335–0251$17.50/0

Accessible online at:
www.karger.com/journals/ore

Dr. Manuel Santos Lacomba
Av. Manolete 18 (4–2º)
E-14005 Cordoba (Spain)
Tel. +34 957 454506, Fax +34 957 412201, E-Mail med000346@nacom.es

Table 1. Clinical features and cytokine levels from patients with uveitis

Patient	Sex	Age years	Cell activity	Location	Etiology	HLA-B27	Patterns	TNF-α, pg/ml	
								AH	serum
1[a]	F	54	Severe	Ant	Ank. spondylitis	Negative	Repeated	16	17
2[a]	M	47	Severe	Ant	Ank. spondylitis	Positive	Repeated	15	42
3[a]	F	29	Severe	Ant	Ank. spondylitis	Positive	Single episode	16	53
4[a]	M	26	Severe	Ant	Ank. spondylitis	Positive	Single episode	16	21
5[a]	F	63	Severe	Ant	Candida	Negative	Single episode	14	16
6[a]	F	16	Mild	Ant	JCA	Negative	Repeated	17	41
7[a]	F	55	Mild	Ant	Behçet's disease	Positive	Repeated	17	70
8[a]	M	49	Severe	Ant	Behçet's disease	Positive	Repeated	16	19
9[a]	M	19	Mild	Ant	GVHD	Negative	Repeated	14	46
10[a]	M	70	Severe	Pan	Toxoplasmosis	Negative	Repeated	17	12
11[a]	M	22	Severe	Pan	Behçet's disease	Negative	Repeated	11	73
12[a]	F	55	Mild	Ant	Ank. spondylitis	Negative	Single episode	14	19
13[b]	M	40	Mild	Ant	Fuchs uveitis	Negative	Single episode	15	12
14[b]	F	52	Severe	Ant	Idiopathic	Positive	Single episode	14	38
15[b]	F	56	Severe	Ant	Idiopathic	Negative	Repeated	12	59
16[b]	F	42	Mild	Ant	Birdshot ret.	Positive	Repeated	17	123
17[b]	M	5	Mild	Ant	Lens induced	Negative	Single episode	16	26
18[b]	M	56	Severe	Ant	Idiopathic	Positive	Repeated	16	12
19[b]	M	19	Severe	Ant	Idiopathic	Negative	Single episode	16	14
20[b]	M	49	Severe	Ant	Idiopathic	Positive	Single episode	16	32
21[b]	F	53	Severe	Ant	Idiopathic	Negative	Single episode	12	12
22[b]	F	33	Mild	Ant	Fuchs uveitis	Negative	Single episode	16	35
23[b]	F	72	Severe	Ant	Idiopathic	Negative	Single episode	14	21
Mean								15.1	35.35
SD								± 1.70	± 26.77

AH = Aqueous humor; Ant = anterior; Pan = panuveitis; JCA = juvenile chronic arthritis; GVHD = graft versus host disease; Ank. = ankylosing; ret. = retinochoroidopathy.

[a] Associated with systemic disease.

[b] Not associated with systemic disease.

pathogenesis of uveitis. On the other hand, studies of TNF-α in serum and mainly in aqueous humor obtained from uveitis patients are few and their results show little agreement.

Many questions have been raised regarding the role and kinetics of TNF-α in human uveitis. In this study we have attempted to determine the behavior of TNF-α and its correlation with clinical characteristics in clinical uveitis by determining it in the aqueous humor and serum of patients with active uveitis.

Methods

A total of 23 patients referred to us with a diagnosis of uveitis as primary process were studied. Patients were included in the study if they had a minimum of 5–10 cells per field (1 mm²) on slit lamp examination, and had a clinical history or characteristics which made it necessary or possible to remove aqueous humor for diagnostic or therapeutic purposes. Pregnant women, HIV patients intravenous drug users and patients receiving local or systemic immunosuppressive treatment were not included in the study. The patients were divided into two categories, those with uveitis associated with systemic diseases, and those with uveitis not associated with systemic disease. The first group comprised 12 patients: 5 had ankylosing spondylitis, 3 Behçet's disease, 1 chronic juvenile iridocyclitis, 1 graft versus host disease, 1 systemic toxoplasmosis, and 1 candida septicemia. The second group comprised 11 patients: 2 had Fuchs' uveitis syndrome, 1 birdshot retinochoroidopathy, 1 lens-induced uveitis, and 7 were idiopathic cases (table 1). Sixteen patients who had been operated on for uncomplicated cataracts served as the control group (table 2). The uveitis group was classified according to the recommendations of the International Group for the Study of Uveitis [14]. We thus took into account laterality, onset (insidious or sudden), duration (short: less than 3 months or chronic: more than 3 months), patterns (single or repeated episodes), visual damage (visual damage

can be mild if visual loss is ≤ 50% of predisease vision, and severe if visual loss is ≥ 50%), location (anterior, intermediate, posterior or panuveitis), and cellular and protein inflammatory activity (all cases who had an activity of 2+ or less were considered mild, and those with an activity of more than 2+ were considered severe). We also took into consideration other factors such as association with systemic disease, previous ocular surgery and the association with the B27 histocompatibility antigen.

Aqueous (0.2–0.3 ml) and peripheral blood (10 ml) samples were taken from each patient before the start of steroid therapy. The aqueous humor was extracted under a surgical microscope via limbic paracentesis using a 27-gauge needle. Before the administration of any systemic drugs, peripheral blood was extracted at the time of collection of the ocular specimen. The aqueous humor was deposited in an Eppendorf tube (Eppendorf, Hamburg, Germany) for subsequent processing. The cells were isolated from the supernatant by centrifugation at 3,500 rpm and both components were stored at −70° C until assay. The supernatant and cells were isolated from the peripheral blood by centrifugation at 3,500 rpm and stored at −70° C until assay.

The Tenets of Declaration of Helsinki were followed. The Research Standards Committee of our center approved this project (Resolution No. 68/95). All patients and controls gave their informed consent after the nature of the study had been fully explained to them.

Detection of TNF-α

Levels of soluble TNF-α were measured in serum and aqueous humor supernatants. A commercial immunoenzymatic ELISA kit (Bender Medsystems, Austria) was used. Serum and aqueous humor samples were diluted 1:20. Briefly, 50 µl of sample or standard controls were added to wells containing absorbed anti-TNF-α monoclonal antibody. After incubation and washing following the protocols of the kits, an HRP-conjugated anti-TNF-α antibody was added and bound to the TNF-α captured by the first antibody. Following incubation any unbound conjugate was removed during a wash step and a substrate solution reactive with HRP (TMB) was added to the wells. A colored product was formed in proportion to the amount of TNF-α present in the sample. The reaction was terminated by addition of acid and the absorbance was measured at 450 nm as the primary wavelength and 620 nm as the reference wavelength. A standard curve was prepared from seven TNF-α standard dilutions and the TNF-α sample concentration was determined. Each determination was carried out in duplicate and the mean of each pair of results was used. The results are expressed in pg/ml and the lower detection limit for TNF-α was 4 pg/ml.

Statistical Analysis

Statistical analyses were performed using SPSS software, version 7.5. Categorical variables were compared by using χ^2 or Fisher's exact tests. Two continuous variables were compared by using Student's t test for independent and related samples when the variables fitted a normal distribution; when they did not we utilized the nonparametric Mann-Whitney U test for independent samples and the Wilcoxon test for related samples. Association between two continuous variables was assessed by means of Pearson's rank correlation coefficient. Multiple linear regression analysis for successive steps was used to assess the relationships among different clinical characteristics of uveitis and the cytokine studied. All statistical tests were two-tailed.

Table 2. Clinical features and cytokine levels from controls

Controls No./sex/age	TNF-α, pg/ml AH	serum
1/M/71	<4.0	11
2/M/47	<4.0	12
3/F/18	<4.0	15
4/F/64	4.5	20
5/M/37	<4.0	17
6/F/51	<4.0	16
7/M/60	<4.0	14
8/F/45	<4.0	12
9/M/54	<4.0	10
10/M/74	<4.0	9
11/F/68	<4.0	11
12/M/22	<4.0	12
13/M/23	4.5	13
14/F/69	<4.0	10
15/F/62	<4.0	15
16/F/59	<4.0	13
Mean ± SD	0.56 ± 1.53	13.13 ± 2.92

AH = Aqueous humor.

Results

The clinical features of the 23 patients with uveitis included in this study and the mean (± SD) values for TNF-α from patients with uveitis and controls are shown in tables 1 and 2. Patients with uveitis and controls did not differ in mean age and sex distribution.

Tumor Necrosis Factor-α

Uveitis patients had higher TNF-α levels in both aqueous humor ($p < 0.001$) and serum ($p < 0.001$) as compared to controls. On the other hand, we observed that serum levels of TNF-α from patients with uveitis (35.35 ± 26.77 pg/ml) were significantly higher than those in the aqueous humor (15.1 ± 1.70 pg/ml) (fig. 1). There was a significant difference in mean sera of TNF-α in repeated episodes when compared with the first episode of uveitis (46.73 ± 33.65 vs. 24.92 ± 12.47 pg/ml, $p < 0.05$). There were no significant differences in mean sera and aqueous humor of TNF-α when the other clinical characteristics of uveitis (laterality, onset, visual damage, location, inflammatory activity, association with systemic disease, previous ocular surgery and association with B27 histocompatibility antigen) were analyzed.

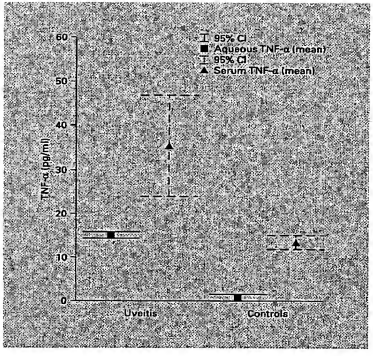

Fig. 1. TNF-α levels in the aqueous humor and serum of patients with uveitis and controls. Mean (central point) and 95% confidence limits (in parentheses) are shown. The assay sensitivity was 4.0 pg/ml.

Table 3. Relationships between TNF-α and clinical characteristics of patients with uveitis

	Aqueous TNF-α		Sera TNF-α	
	r	p	r	p
Inflammatory activity	−0.3897	0.5620	−0.2542	0.2133
Previous ocular surgery	0.3191	0.1553	−0.1591	0.4378
Duration	−0.1402	0.5262	0.2625	0.1931
Age	−0.1516	0.4672	−0.2025	0.3130
Sex	−0.5980	0.7761	0.2048	0.1564
Etiology	0.0815	0.6962	−0.1208	0.5747
HLA-B27	0.3859	0.0689	0.2909	0.1474
Onset	0.4017	0.0523	0.2027	0.3264
Laterality	0.2381	0.2505	−0.0194	0.9263
Visual damage	−0.8094	0.6979	−0.1177	0.6236
Pattern	0.1339	0.5198	0.4150	0.0489*

* $p < 0.05$.

Correlation Studies

When TNF-α was analyzed for clinical characteristics of the patients with uveitis by the regression test for successive steps, an association was observed between recurrent uveitis and serum levels of TNF-α ($r = 0.4150$; $p = 0.0489$) (table 3). No relationships were found between the age of the patients and TNF-α or the other clinical characteristics of uveitis and TNF-α.

Discussion

TNF-α has been widely studied in the EIU experimental model. De Vos et al. [10] thus refer to an early release of TNF-α in aqueous humor, prior to the appearance of any clinical symptom of uveitis, suggesting that this cytokine may be an early mediator in the pathogenesis of EIU. While studies of TNF-α using experimental models of uveitis are numerous [15–17], studies of TNF-α in ocular material obtained from uveitis patients are scant and demonstrate little agreement. Our study shows that the aqueous humor and sera from patients with uveitis have higher levels of TNF-α than those from controls. Previous reports have demonstrated that the aqueous humor from patients with uveitis contains TNF-α. Thus, Palexas et al. [6] reported 1 patient with sympathetic ophthalmia in whom they detected elevated ocular and systemic levels of TNF, and Franks et al. [8] found detectable levels of TNF-α in only one of five samples of vitreous humor from uveitis patients. Likewise, Rahi and Al-Kaff [2] found that serum levels of TNF-α are increased in patients with retinal vasculitis but not in other uveitis subgroups. These data support our present findings and reinforce the idea that TNF-α may be a mediator in ocular inflammation. However, Feys et al. [18] were unable to detect TNF-α in either serum or aqueous humor of patients with uveitis and endophthalmitis, and Lee et al. [7] did not observe changes in sera TNF-α levels of patients with uveitis. It is difficult to interpret these discrepancies; the lack of agreement may be due to the different laboratory techniques employed, a failure to take into account kinetic factors inherent in the evolution of the disease, or to differences in the etiological classification of the syndromes involved. Therefore, further studies are needed in more specific uveitis subgroups.

It was interesting that our patients with uveitis showed higher levels of TNF-α in serum than in the aqueous humor. This suggests the existence of a leakage from the blood to the aqueous humor rather than local production. Although the significance of these higher levels of serum TNF-α is unknown, it may be related, as in some experimental models of uveitis, to the initiation of a regulatory

and protective response at the systemic level. De Vos et al. [19] have demonstrated that systemic treatment with anti-TNF-α antibodies in rats provokes an exacerbation of EIU, suggesting that serum TNF-α may have a protective effect by inhibiting the migration of polymorphonuclear cells to the focus of inflammation and that TNF-α could induce a generalized activation of adhesion molecules at the systemic level, which would further avoid the local inflow of inflammatory cells.

Regarding the behavior of this cytokine according to the different clinical characteristics of the uveitis (age, sex, laterality, onset, patterns, visual damage, location, inflammatory activity, association with systemic disease, previous ocular surgery, and association with B27 histocompatibility antigen) we observed that patients with recurrent uveitis had higher levels of serum TNF-α than those who were suffering the first episode of their disease. Because the existence of recurrent uveitis worsens the visual prognosis, the high values of serum TNF-α found in the recurrent uveitis group may have a prognostic value, although more studies will be needed to confirm this hypothesis. On the other hand, when we try to differentiate the behavior of serum TNF-α according to whether the uveitis was or was not associated with systemic disease, it is interesting to point out that although the serum TNF-α levels in both subgroups were significantly higher than in controls, no difference was observed between serum TNF-α levels of uveitis patients with systemic disease and those of the uveitis patients without systemic disease. These findings suggest that the inflammatory phenomena may not be restricted to the eye only, but that in fact at least some forms of idiopathic uveitis may represent systemic autoimmune diseases with ocular manifestations only.

References

1 Ongkosuwito JV, Feron EJ, van Doornik CE, Van der Lelij A, Hoyng CB, La Heij EC, Kijlstra A: Analysis of immunoregulatory cytokines in ocular fluid samples from patients with uveitis. Invest Ophthalmol Vis Sci 1998;39: 2659–2665.

2 Rahi AH, Al-Kaff A: Cytokine profile in uveitis: A point prevalence study; in Whitcup SM, Nussenblatt RB, Caspi RR, Gery I (eds): Advances in Ocular Immunology. International Congress Series. Amsterdam, Elsevier Science, 1994, pp 369–372.

3 Calder VL, Shaer B, Muhaya M, Mclauchlan M, Pearson RV, Jolly J, Towler HM, Lightman S: Increased CD4+ expression and decreased IL-10 in the anterior chamber in idiopathic uveitis. Invest Ophthalmol Vis Sci 1999;40: 2019–2024.

4 Murray PI, Hockzema R, Van Haren MAC, De Hon FD, Kijlstra A: Aqueous humor interleukin-6 levels in uveitis. Invest Ophthalmol Vis Sci 1990;31:917–920.

5 Norose K, Yano A, Wang XC, Tokushima T, Umihira J, Seki A, Nohara M, Segawa K: Dominance of activated T cells and interleukin-6 in aqueous humor in Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci 1994;35:33–39.

6 Palexas GN, Sussman G, Welsh NH: Ocular and systemic determination of IL-1 beta and tumour necrosis factor in a patient with ocular inflammation. Scand J Immunol Suppl 1992; 11:173–175.

7 Lee SJ, Li Z, Sherman B, Foster CS: Serum levels of tumor necrosis factor-alpha and interleukin-6 in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 1993;34:3522–3525.

8 Franks WA, Limb GA, Standford MR, Ogilvie J, Wolstencroft RA, Chignell AH, Dumonde DC: Cytokines in human intraocular inflammation. Curr Eye Res 1992;11:187–191.

9 Tracey KJ: Tumor necrosis factor; in Remick DG, Friendland JS (eds): Cytokines in Health and Disease. New York, Dekker, 1997, pp 223–239.

10 De Vos AF, Van Haren MAC, Verhagen C, Hoekzema R, Kijlstra A: Kinetics of intraocular tumor necrosis factor and interleukin-6 in endotoxin-induced uveitis in the rat. Invest Ophthalmol Vis Sci 1994;35:1100–1106.

11 Kasner L, Chan CC, Whitcup SM, Gery I: The paradoxical effect of tumor necrosis factor alpha (TNF-α) in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 1993;34:2911–2917.

12 Rosenbaum JT, Howes EL, Rubin RM, Samples JR: Ocular inflammatory effects of intravitreally-injected tumor necrosis factor. Am J Pathol 1988;133:47–53.

13 De Vos AF, Van Haren MAC, Verhagen C, Hoekzema R, Kijlstra A: TNF-induced uveitis in the Lewis rat is associated with intraocular interleukin 6 production. Exp Eye Res 1995; 60:199–207.

14 Bloch-Michel E, Nussenblatt RB: International uveitis study group recommendations for the evaluation of intraocular inflammatory disease. Am J Ophthalmol 1987;103:234–235.

15 Kogiso M, Tanouchi Y, Mimura Y, Nagasawa H, Himeno K: Endotoxin-induced uveitis in mice. 1. Induction of uveitis and role of T lymphocytes. Jpn J Ophthalmol 1992;36:281–290.

16 Brito BE, O'Rourke LM, Pan Y, Anglin J, Planck SR, Rosenbaum JT: IL-1 and TNF-α receptor-deficient mice show decreased inflammation in an immune complex model of uveitis. Invest Ophthalmol Vis Sci 1990;31:917–920.

17 Dick AD, Duncan L, Hale G, Waldmann H, Isaacs J: Neutralizing TNF-alpha activity modulates T-cell phenotype and function in experimental autoimmune uveoretinitis. J Autoimmun 1998;11:255–264.

18 Feys J, Emond JP, Salvanet-Bouccara A: Interleukine-6 et autres cytokines dans l'humeur aqueuse au cours d'uvéites et d'endophthalmies. J Fr Ophtalmol 1994;17:634–639.

19 De Vos AF, Van Haren MAC, Verhagen C, Hoekzema R, Kijlstra A: Systemic anti-tumor necrosis factor antibody treatment exacerbates endotoxin-induced uveitis in the rat. Exp Eye Res 1995;61:667–675.